# *Review article*

# Clinical mitochondrial genetics

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The last decade has been an age of enlightenment as far as mitochondrial pathology is concerned. Well established nuclear genetic diseases, such as Friedreich's ataxia,[1 2] Wilson disease,[3] and autosomal recessive hereditary spastic paraplegia,[4] have been shown to have a mitochondrial basis, and we are just starting to unravel the complex nuclear genetic disorders which directly cause mitochondrial dysfunction (table 1). However, in addition to the 3 billion base pair nuclear genome, each human cell typically contains thousands of copies of a small, 16.5 kb circular molecule of double stranded DNA (fig 1). Mitochondrial DNA (mtDNA) accounts for only 1% of the total cellular nucleic acid content. It encodes for 13 polypeptides which are essential for aerobic metabolism and defects of the mitochondrial genome are an important cause of human disease.[92 93] Since the characterisation of the first pathogenic mtDNA defects in 1988,[5 13] over 50 point mutations and well over 100 rearrangements of the mitochondrial genome have been associated with human disease[94 95] (http://www.gen.emory.edu/mitomap.html). These disorders form the focus of this article. (*J Med Genet* 1999;**36**:425–436)

Many mtDNA diseases present with neurological symptoms and signs, but the clinical features are often non-specific and diffuse, and mitochondrial diseases have frequently been misdiagnosed when only clinical abnormalities are considered. MtDNA defects may present to physicians in any specialty and many of these disorders appear to be sporadic. It is precisely because of the diverse clinical features associated with pathogenic mtDNA defects that, not unreasonably, mitochondrial disease enters the differential diagnosis of so many conditions.[96] This marked clinical heterogeneity creates difficulties for the clinician. Unlike nuclear genetic defects, which can often be diagnosed with a simple blood test, the investigation of mitochondrial disease is far more complex, and molecular genetic studies play only one part in the multidisciplinary approach that should be used for these patients.[97]

This articles focuses on three fundamental questions of clinical mitochondrial genetics. (1) When should we look for mitochondrial disease? (2) How should we investigate patients with suspected mitochondrial disease? (3) How should we manage patients with mtDNA disease?

## The clinical features of mtDNA disease

Patients with mtDNA disease fall into three main categories. In the minority of cases, it is relatively straightforward to identify that a clinical syndrome involves mitochondrial dysfunction and these constitute the first category. Classically, patients with MELAS (*M*itochondrial *E*ncephalomyopathy with *L*actic *A*cidosis and *S*troke-like episodes) proceed through their normal motor and cognitive milestones but their stature usually remains short. They often develop bilateral deafness in their late childhood or teenage years, and they present with diabetes, seizures, stroke-like episodes, and an encephalopathy in their third or fourth decade.[98] Not all MELAS patients, however, fit this pattern and diabetes and deafness may be the only features,[99] and a cardiomyopathy may be missed.[100] As another example, patients with *L*eber *H*ereditary *O*ptic *N*europathy (LHON) are more commonly male, and they usually present in their second or third decade with acute or subacute bilateral visual failure, which may recover to some degree in the following months.[101] Although these presentations are "classical", it cannot be stressed too heavily that mtDNA diseases are phenotypically diverse. Thus, the first feature of MELAS may be a stroke-like episode in late middle age,[102 103] and LHON may present in the seventh decade or it may have an atypically insidious onset.[104]

There are other "classical" mitochondrial disorders. External ophthalmoplegia is a relatively common feature of patients with a mitochondrial encephalomyopathy.[97] This abnormality may present in the second decade in association with ataxia, bilateral deafness, a cardiac conduction defect (fig 2), and high CSF protein (as in Kearns-Sayre syndrome, fig 3). More often, it occurs in isolation or with ptosis and a mild proximal myopathy (CPEO, or *C*hronic *P*rogressive *E*xternal *O*phthalmoplegia).[6] Two other syndromes warrant a mention, a neuropathy with ataxia and a pigmentary retinopathy and ataxia is suggestive of NARP (*N*eurogenic weakness *A*taxia with *R*etinitis *P*igmentosa),[23] and myoclonic epilepsy with ataxia and a myopathy are promi-

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*Table 1 Mitochondrial respiratory chain diseases*

**(A) Primary mitochondrial DNA mutations**

(1) Rearrangements

(a) Single deletions
CPEO (MIM 165130)[5 6]
Kearns-Sayre syndrome (MIM 165100)[7]
Pearson syndrome[8]
Diabetes and deafness[9]

(b) Multiple deletions
Encephalomyopathy[10]
Recurrent myoglobinuria[11]
SANDO[12]

(2) Point mutations

(a) Missense mutations (coding region)
LHON (MIM 535000)
G11778A ND5[13]
G3460A ND1[14]
T14484C ND5[15]
Optic atrophy and dystonia
A11696G ND4[16]
G14459A ND6[17]
T14596A ND6[16]
MELAS
T9957C COX III[18]
G13515A ND5[19]
Encephalomyopathy and myopathy
G9952A COX III[20]
NARP/Leigh disease/striatal necrosis
T3308C ND1[21]
T8851C ATPase6[22]
T8993G/C ATPase6[23 24]
T9176C ATPase6[25 26]

(b) Transfer RNA gene
MELAS (MIM 251910)
G1642A tRNA$^{Val}$ [27]
A3243G tRNA$^{Leu(UUR)}$ [28]
A3252G tRNA$^{Leu(UUR)}$ [29]
A3260G tRNA$^{Leu(UUR)}$ [30]
T3271C tRNA$^{Leu(UUR)}$ [31]
T3291C tRNA$^{Leu(UUR)}$ [32]
A5814G tRNA$^{Cys}$ [33]
MERRF (MIM 254775)
A8344G tRNA$^{Lys}$ [34]
T8356C tRNA$^{Lys}$ [35]
G8363 tRNA$^{Lys}$ [36]
MERRF/MELAS overlap
T7521C tRNA$^{Ser(UCN)}$ [37]
T8356C tRNA$^{Lys}$ [38]
Diabetes and deafness
A3243G tRNA$^{Leu(UUR)}$ [39]
T12258AtRNA$^{Ser(AGY)}$ [40]
Cardiomyopathy
A3243G tRNA$^{Leu(UUR)}$ [41]
A3254G tRNA$^{Leu(UUR)}$ [42]
A3260G tRNA$^{Leu(UUR)}$ [43]
C3303T tRNA$^{Leu(UUR)}$ [44]
A4269G tRNA$^{Ille}$ [45]
A4295G tRNA$^{Ille}$ [46]
A4300G tRNA$^{Ille}$ [47]
C4320T tRNA$^{Ille}$ [48]
G8363A tRNA$^{Lys}$ [49]
T9977C tRNA$^{Gly}$ [50]
CPEO
A3243G tRNA$^{Leu(UUR)}$ [51]
A3251G tRNA$^{Leu(UUR)}$ [52]
C3256T tRNA$^{Leu(UUR)}$ [53]
T4274C tRNA$^{Ille}$ [54]
T4285C tRNA$^{Ille}$ [55]
G4298A tRNA$^{Ille}$ [56]
A5692G tRNA$^{Asn}$ [57]
G5703A tRNA$^{Asn}$ [57]
T12311C tRNA$^{Leu(CUN)}$ [58]
G12315A tRNA$^{Leu(CUN)}$ [59]
Pure myopathy
A606G tRNA$^{Phe}$ [60]
A3243G tRNA$^{Leu(UUR)}$ [51]
T3250C tRNA$^{Leu(UUR)}$ [61]
A3302G tRNA$^{Leu(UUR)}$ [62]
T4409C tRNA$^{Met}$ [63]
G5521A tRNA$^{Trp}$ [64]
A8344G tRNA$^{Lys}$ [34]
A12330G tRNA$^{Leu(CUN)}$ [65]
C15990T tRNA$^{Pro}$ [66]
Myopathy and diabetes
A14709G tRNA$^{Glu}$ [67]
Encephalomyopathy
G1606A tRNA$^{Val}$ [68]
T10010C tRNA$^{Glu}$ [69]
G15915A tRNA$^{Trp}$ [70]
Dementia and chorea
G5549A tRNA$^{Trp}$ [71]
Gastrointestinal dysmotility
A3243G tRNA$^{Leu(UUR)}$ [72]
G8313A tRNA$^{Lys}$ [73]
Leigh syndrome
G1644T tRNA$^{Val}$ [74]
Non-syndromic deafness
A7455G tRNA$^{Ser(UCN)}$ [75]
Acquired sideroblastic anaemia
G12301A tRNA$^{Leu(CUN)}$ [76]
(c) Ribosomal RNA gene
Non-syndromic/aminoglycoside induced deafness
G1555A 12S rRNA[77]

**(B) Nuclear gene mutations**

(1) Nuclear gene defects indirectly affecting mitochondrial function through mtDNA
Autosomal dominant CPEO with multiple mtDNA deletions (10q23.3-q24.3; 3p14.1-21.2)[78 79]
Mitochondrial neurogastrointestinal encephalomyopathy (thymidine phosphorylase deficiency — mutations in thymidine phosphorylase gene 22q13.32-qter)[80]

(2) Nuclear genetic disorders of the mitochondrial respiratory chain
Multisystem disease (complex I deficiency — mutations in AQDQ subunit on chr 5)[81]
Leigh syndrome (complex I deficiency — mutations in NDUFS8 subunit)[82]
Optic atrophy and ataxia (complex II deficiency — mutations in Fp subunit of SDH on chr 3)[83]
Leigh syndrome (complex II deficiency — mutations in Fp subunit of SDH on chr 3)[84]
Leigh syndrome (complex IV deficiency — mutations in SURF I gene 9q1)[85 86]
Benign infantile myopathy with COX deficiency[87]
Fatal infantile myopathy with COX deficiency[88]

nent features of MERRF (*M*yoclonic *E*pilepsy with *R*agged *R*ed *F*ibres).[34]

The second group of patients includes those who have a constellation of clinical features which are highly suggestive of mitochondrial disease, but who do not neatly fall into a specific syndrome category (table 2). Thus, short stature in association with bilateral sensorineural deafness, ophthalmoplegia with ptosis, or a history of migraine and diabetes are three clinical presentations that suggest a mitochondrial aetiology. A meticulous neurological examination is essential in all patients with suspected mitochondrial disease. The combination of any of the above features with clinical evidence of a myopathy and central neurological signs places a mtDNA defect high on the list of differential diagnoses. However, mtDNA disease may mimic other inherited neurological disorders, and patients with mtDNA defects often undergo numerous autosomal genetic tests before the correct diagnosis is made. For example, mitochondrial disease may present as a spinocerebellar syndrome[142] and a mtDNA defect should be considered in patients who test negative for the known spinocerebellar ataxia (SCA) mutations (particularly if they have an ophthalmoplegia and if there are no documented paternal transmissions). Focal[143] or generalised[144] dystonia with optic atrophy may be confused with the early stages of Huntington's disease. Usher syndrome (pigmentary retinopathy with deafness) shares clinical features with mtDNA disease[145] and patients with mtDNA defects may present with a motor and sensory neuropathy similar to Charcot-Marie-Tooth disease.[142] Finally, we have seen a number of patients who were intially diagnosed as "congenital myasthenia". This list of "missed" diagnoses of mitochondrial diseases is by no means complete, but it serves to illustrate the daunting scope of the situation that confronts the clinician.
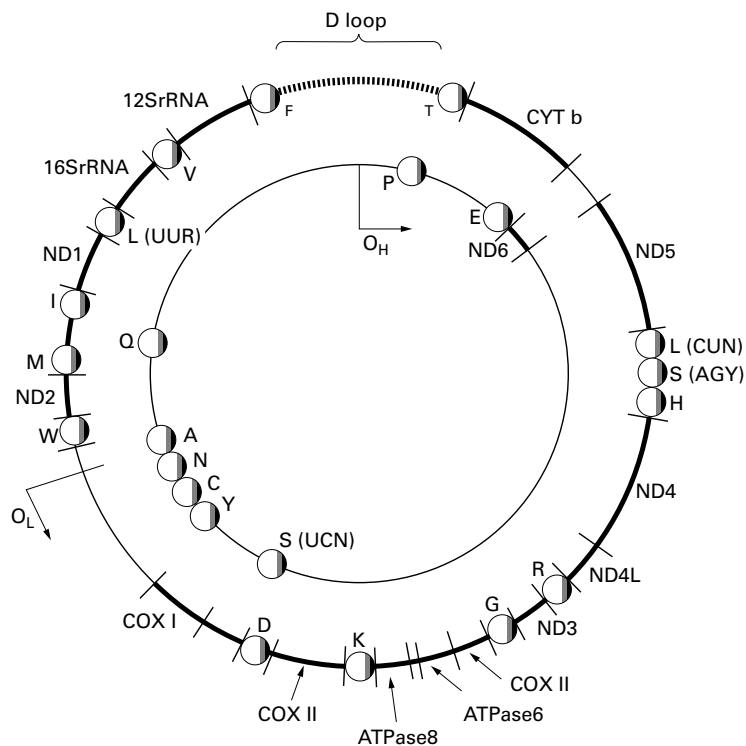
*Figure 1   The mitochondrial genome and basic mitochondrial genetics. Human mitochondrial DNA (mtDNA) is a 16.569 bp circle of double stranded DNA that encodes 13 essential respiratory chain protein subunits. The ND1-ND6 and the ND4L genes encode seven complex I subunits (NADH-ubiquinone oxidoreductase), the CYTb gene encodes one complex III subunit (ubiquinol-cytochrome c oxidase oxidoreductase), the COX I to COX III genes encode three complex IV subunits (cytochrome c oxidase), and the ATPase6 and ATPase8 genes encode for complex V subunits (ATP synthase). Interspaced between the protein encoding genes are two ribosomal RNA genes (12S and 16S rRNA), and 22 transfer RNA genes that provide the necessary RNA components for the mitochondrial translation machinery. The protein subunits of the mitochondrial ribosomes are encoded by nuclear genes. The 1.1 kb non-coding region (called the displacement loop or D loop) is important for the generation of mtDNA transcripts and the replication of mtDNA. $O_H$ is the origin of heavy (outer) strand replication, $O_L$ is the origin of light (inner) strand replication. MtDNA differs from nuclear DNA in a number of ways. (1) Each mononuclear human cell contains between 1000 and 100 000 copies of mtDNA. (2) MtDNA molecules very rarely, if ever, undergo intramolecular recombination. (3) The mitochondrial matrix is rich in mutagens that are generated during electron transfer and oxidative phosphorylation, but mtDNA is not protected by histones, and mitochondria are relatively deficient in DNA repair mechanisms, As a result, mtDNA accumulates mutations at a much faster rate than nuclear DNA. Mitochondrial diseases may result from either mutations of mtDNA or nuclear DNA. Despite the complexities of the mitochondrial genome, its small size makes it amenable to molecular genetic analysis. As a result, over 100 different mtDNA rearrangements and over 50 mtDNA point mutations have been associated with disease. The map position of mtDNA mutations is designated with reference to the L strand of the Cambridge reference sequence.[89] Because the tRNA genes are interspersed throughout the genome, mtDNA deletions usually involve a tRNA gene, resulting in a protein translation defect, and causing Kearns-Sayre syndrome, chronic progressive external ophthalmoplegia, or diabetes and deafness (table 1). Point mutations may affect protein coding genes (missense), ribosomal genes, or tRNA genes. Missense mutations may be homoplasmic (all of the mtDNA molecules within the cell are mutated and identical), and cause disorders such as Leber hereditary optic neuropathy (LHON), or neurogenic weakness with ataxia and pigmentary retinopathy through a direct effect on the protein encoding gene. Mutations which involve tRNA genes alter mitochondrial function through a number of different mechanisms that include abnormal transcription[90] and abnormal translation.[91] These mutations result in a wide variety of disorders such as mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS), or myoclonic epilepsy with ragged red fibres (MERRF). Patients who harbour these mutations usually have a mixture of mutated and normal mtDNA (heteroplasmy). In vitro studies have shown that a biochemical defect of mitochondrial function is only expressed when a critical percentage level of mutated mtDNA is exceeded. This threshold varies from tissue to tissue and is, in part, related to the dependence of a particular organ upon oxidative metabolism. This variability, coupled with differences in the percentage level of mutated mtDNA within subjects, contributes to the phenotypic variability which is characteristic of mitochondrial tRNA gene disorders. Nuclear gene defects are a major cause of mitochondrial disease, accounting for up to 30% of adult cases and an even higher proportion of paediatric cases. In the majority of subjects, a nuclear gene defect is inferred, whether because of the pattern of respiratory chain involvement, or the distribution of the mitochondrial defect in single muscle fibres. However, nuclear gene mutations have recently been identified in patients with Leigh syndrome resulting from complex I and complex IV deficiency, and point mutations in the succinate dehydrogenase gene cause autosomal dominant optic atrophy and ataxia.*

The last of the three main groups is the most difficult to define. Clinicians are seeing an increasing number of patients who are referred with unexplained symptoms and signs who, surprisingly, turn out to have mtDNA disease (table 2). As one example, mtDNA defects cause a small, but significant proportion of strokes in the very young.[146 147] Furthermore, mitochondrial diseases often have a non-neurological presentation, with features such as a hypertrophic cardiomyopathy[41 43 44 100 148] and renal tubular disease.[130 131 149 150] Endocrine abnormalities are common and hypoparathyroidism,[29 121 122 151] adrenal insufficiency,[152 153] and diabetes[99] have also been widely documented.[154] In fact, between 0.5 and 1% of diabetics harbour a causative mtDNA mutation[154] and even this prevalence is probably an underestimate because the total number of mtDNA mutations that can cause diabetes is not known. Gastrointestinal abnormalities are also common in mitochondrial patients, although they rarely volunteer this information spontaneously.[96] Recurrent vomiting has been mistaken for anorexia nervosa and a gastrectomy was carried out before the correct mitochondrial aetiology was established.[72 126] Mitochondrial dysphagia may lead to a percutaneous gastrostomy before the age of 20[96] and both constipation and diarrhoea are also common. It is unclear at present whether these features result from a visceral myopathy or a myenteric plexus neuropathy.

Although deafness is a common feature of mtDNA disease, one particular mutation deserves a special mention. In a large study of 70 Spanish families with deafness, 27% were found to harbour a homoplasmic point mutation at mtDNA nucleotide 1555. This mutation was associated with both congenital and late onset deafness, and the penetrance was enhanced by exposure to aminoglycosides.[105] The identification of this mutation should make it possible to prevent, or at least to delay, the onset of deafness in susceptible subjects by avoiding exposure to aminoglycosides. This problem is potentially of major importance, particularly in countries where streptomycin is frequently used in the treatment of tuberculosis.[155]

The list of clinical features associated with mtDNA defects is seemingly endless. How then should we approach the problem in the general genetics clinic? It is sometimes possible to elicit a maternal family history, but unfortunately this is often not the case. A history of recurrent spontaneous abortion and early neonatal death is not uncommon in pedigrees of mtDNA disease, but even with an extended geneaology, mitochondrial inheritance may be confused with dominant, recessive, or sex linked inheritance.[145] Taking a family history is made all the more difficult when we consider the relatively non-specific features of some mitochondrial diseases. How many families have relatives with diabetes, strokes, heart disease, and migraine? From a practical point of view, it is sensible to consider a mitochondrial aetiology in any case of multisystem disease,
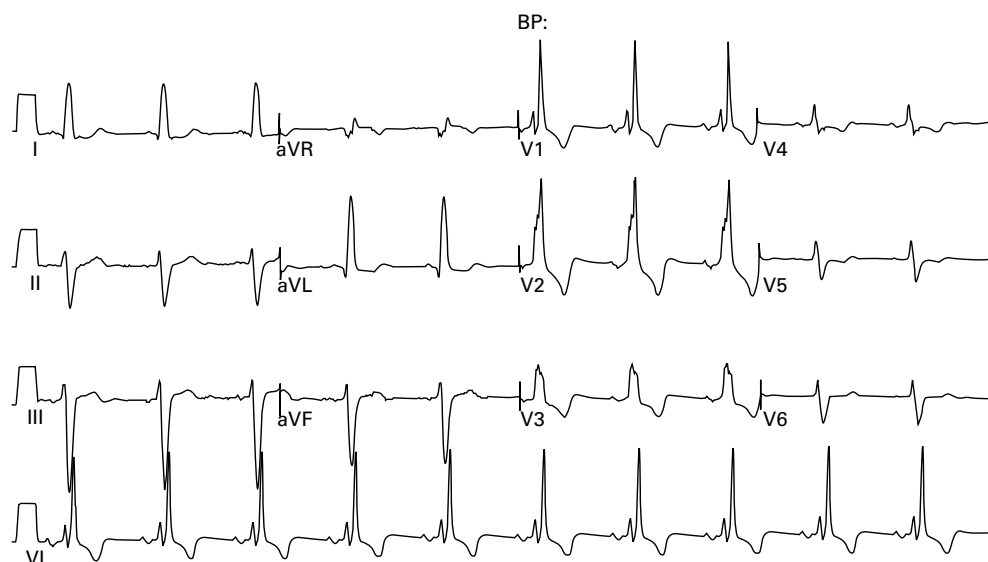
*Figure 2　Twelve lead electrocardiogram from a patient with Kearns-Sayre syndrome showing the broad QRS complexes of bifasicular block (right bundle branch block with left anterior fasicular block).*

particularly if it involves the central nervous system.

In our experience, the overt presentation of mitochondrial disease is less common in children than in adults and it may be quite different. In the neonatal period, mitochondrial disease can present as a metabolic encephalopathy with hepatic and cardiac failure, as well as an associated lactic acidosis.[137] Most of these babies die, but it is essential to make an accurate diagnosis because this presentation may mimic X linked pyruvate dehydrogenase complex (PDC) deficiency, or a potentially treatable metabolic disease such as biotinidase deficiency. More accurate diagnosis of a

mitochondrial aetiology should thus enable genetic counselling and prenatal diagnosis.[156] Older children may present with an encephalopathy (seizures and coma), prominent brain stem signs (bizarre eye movements and ataxia), and extrapyramidal disease (dystonia and chorea) suggestive of Leigh syndrome (also called subacute necrotising encephalopathy). These



*Figure 3　Kearns-Sayre syndrome. Note the bilateral ptosis, myopathic facies, and the hearing aid. (Photograph reproduced with permission.)*

*Table 2　Clinical features of mitochondrial disease*

Neuromuscular (for review see ref 97)
　External ophthalmoplegia and ptosis[6 7]
　Sensorineural deafness[105 106]
　Myopathy[51 65]
　Migraine[107 108]
　Seizures/myoclonus[109 110]
　Encephalomyopathy[28 111]
　Dementia[71 111]
　Ataxia[112]
　Stroke-like episodes[113]
　Parkinsonism/dystonia[114]
　Spastic paraparesis[115]
　Peripheral neuropathy[116]
　Rhabdomyolysis[60]
　Optic atrophy[109]
　Pigmentary retinopathy[109]
Cardiac
　Hypertrophic cardiomyopathy[43]
　Accessory pathways[117 118]
　Heart block[119]
Endocrine
　Diabetes mellitus[99 120]
　Hypoparathyroidism[121 122 123]
　Hypogonadism[124]
　Infertility[125]
Gastrointestinal
　Dysphagia[96]
　Cyclical vomiting[72 126]
　Pseudo-obstruction[127 128]
Renal
　Aminoaciduria[129]
　Renal tubular dysfunction[130]
　Tubulointerstitial disease[131]
　Toni-Fanconi-Debre syndrome[132 133]
　Barrter syndrome[134]
Haematological
　Sideroblastic anaemia[135]
　Pancytopenia[135]
Pancreas and liver
　Exocrine pancreatic failure[136]
　Hepatocellular failure[137]
Pyschiatric
　Depression[138 139]
　Psychotic illness[140]
Dermatological
　Lipomatosis[141]

ease may also present with sideroblastic anaemia, pancytopenia, and exocrine pancreatic failure (Pearson syndrome).[135] Finally, children with mtDNA defects may present with non-specific features such as failure to thrive and developmental delay. It is often difficult to decide when to investigate these patients, and because the most revealing investigations are invasive, other treatable causes should be ruled out before embarking on a series of investigations for mtDNA disease.

## The investigation of suspected mitochondrial DNA disease

Mitochondrial medicine is a "young", but increasingly important clinical specialty. The biochemical and molecular genetic tests help to confirm the clinical diagnosis. It would be both inappropriate and potentially misleading to send samples for mtDNA analysis as part of a diagnostic "fishing exercise", because the same phenotype can be caused by many different mutations. As one example, at least 11 different pathogenic mutations have been identified in the mitochondrial tRNA[leu(UUR)] gene, several of which cause MELAS. It is important, therefore, to follow a structured investigatory pathway that is driven by the family history and the findings from clinical examination (fig 5). The investigations fall into two main groups: general investigations that are used to accumulate evidence of the pattern and nature of the different organs involved in mitochondrial disease, and specific investigations that seek biochemical/molecular genetic evidence of a mitochondrial abnormality.

GENERAL CLINICAL INVESTIGATIONS

MtDNA disease is often considered after many other diagnoses have been excluded. For this

*Figure 4   CT scan of the brain showing bilateral basal ganglia calcification in a patient harbouring the A3243G (MELAS) mutation.*

children often have characteristic neuroimaging on CT, with hypodensities within the basal ganglia.[111] Leigh syndrome may result from a nuclear gene defect (usually autosomal recessive[81 82 85 86]), but occasionally X linked PDC deficiency[156] or a mtDNA defect (a point mutation in the mitochondrial ATPase6 gene).[157 158] Children with mitochondrial dis-
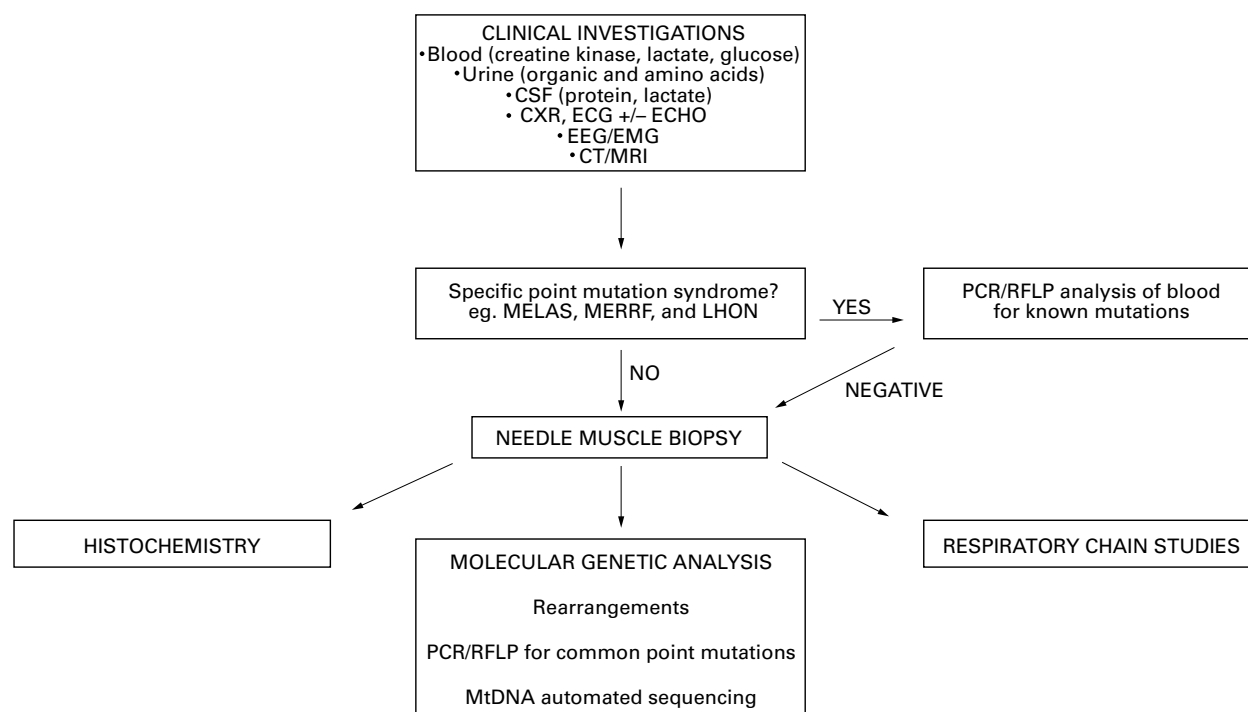
*Figure 5   The investigation of mitochondrial DNA disease.*

reason, patients have usually had many general diagnostic tests by the time they are seen by a specialist. It is essential to assess cardiac function (ECG and echocardiography) and to carry out an oral glucose tolerance test because of the high prevalence of cardiac complications and diabetes in patients with mtDNA disease. Patients with a mitochondrial respiratory chain defect sometimes, but not exclusively, have a raised blood lactate level.[111] As far as mitochondrial disease goes, therefore, this test is neither specific nor sensitive. There are many causes of a blood lactic acidosis, and many patients with mtDNA defects have a normal blood lactate (particularly when presenting as adults). A raised CSF lactate is a more specific test for mitochondrial disease, if there is central neurological involvement, although many non-mitochondrial neurological conditions result in a CSF lactic acidosis (for example, seizures, migraine, thromboembolic stroke). The serum creatine kinase may be raised, but it is often normal,[111] even when the patient has a proximal myopathy. Urine organic and amino acids may also be abnormal in patients with mitochondrial dysfunction.[129] Every patient with seizures or cognitive decline should have an electroencephalogram (EEG) which may show evidence of seizure activity or diffuse slow waves suggestive of a subacute encephalopathy. They should also have a brain CT scan, which may show calcified basal ganglia (fig 4) or bilateral hypodensities and atrophy, which are valuable diagnostic indicators of mitochondrial disease.[159] MRI may also be indicated, particularly in a patient with brain stem signs or stroke-like episodes. The abnormalities are often non-specific (cerebral and cerebellar atrophy),[160 161] but they can be indicative of a mtDNA defect.[162]

SPECIFIC INVESTIGATIONS: HISTOCHEMISTRY, BIOCHEMISTRY, AND MOLECULAR GENETICS

A basic understanding of mitochondrial genetics is essential for the physician who is investigating a patient with suspected mtDNA disease (fig 1). Each human cell contains between 1000 and 100 000 copies of mtDNA. The vast majority of these molecules are identical (homoplasmy) in healthy subjects. Pathogenic mtDNA mutations fall into two groups: rearrangements (deletions and duplications) and point mutations. Patients with pathogenic mtDNA defects usually harbour a mixture of mutated and wild type molecules within each cell (heteroplasmy).[93] At the cellular level, the proportion of mutated mtDNA (mutation load) determines phenotypic expression, because the load must exceed a critical threshold level before the cell develops a biochemical defect of the respiratory chain (typically >85% mutated mtDNA).[163] Part of the clinical variability associated with a particular mtDNA mutation can be attributed to differences in the overall mutation load among people.[117 164] However, the level of heteroplasmy not only varies between people, but it also varies within them. Therefore, unlike nuclear genetic defects which are present within every cell (with the notable, but rare exception of somatic mosai-

cism), the amount of mutant mtDNA varies from cell to cell and from organ to organ. This complexity provides a further explanation for the diversity of mtDNA diseases, but it can also make the investigation of these patients particularly difficult.

For some mitochondrial diseases, it is possible to obtain an accurate diagnosis with a simple molecular genetic screen (fig 5). For example, 95% of patients with LHON harbour one of three mtDNA point mutations (A11778G,[13] A3460G,[165] and T14484C of the mtDNA L strand[15]).[166] These patients have very high levels of mutated mtDNA in blood, and it is therefore appropriate to send blood for molecular genetic analysis by PCR/RFLP analysis. Similarly, the level of mutated mtDNA in blood is very high for most MERRF patients who harbour a point mutation in the lysine tRNA gene at position 8344 and, again, the diagnosis can be made from a blood sample.[164] In contrast, patients with the A3243G MELAS mutation often have low levels of mutated mtDNA in blood. As a result, it can be estimated that at least 1 in 20 MELAS patients will be missed by the conventional PCR/RFLP approach.[167] The mtDNA deletions that cause CPEO and the Kearns-Sayre syndrome are also usually not detectable in blood. The results of such simple mtDNA genetic screens, therefore, must be interpreted with extreme caution. If clinical suspicion was strong enough to warrant the blood test, then patients with a negative result should be investigated further. This usually involves a skeletal muscle biopsy (fig 5). To the unfamiliar, this procedure may seem excessively invasive, particularly if the symptoms and signs are minor. However, needle biopsy is relatively benign and we routinely perform this procedure under local anaesthetic on the neurology ward. The procedure takes approximately 45 minutes from start to finish, is extremely well tolerated, and without major complication (in over 400 biopsies over the last two years).

The analysis of muscle forms the cornerstone of the investigation of patients with suspected mitochondrial disease. Histochemical analysis may show subsarcolemmal accumulations of mitochondria (so called ragged red fibres or RRFs). However, these RRFs are a relatively non-specific finding, because they are seen in a variety of muscle diseases[115] and in muscle taken from healthy athletes. Similarly, electron microscopy may show abnormal mitochondria with paracrystalline inclusions but these are also relatively non-specific.[168] In contrast, muscle histochemistry may also show cytochrome c oxidase (COX, complex IV) deficient fibres which indicate mitochondrial dysfunction. Respiratory chain complex assays may also show a deficiency.[169] Either of these two abnormalities confirm that a patient has mitochondrial disease, and the pattern of the abnormality provides an invaluable guide to the molecular geneticist for additional analyses.

The mitochondrial respiratory chain has an unusual genetic composition (fig 1). Thirteen essential polypeptides are synthesised from genes within the mitochondrion itself, but the

majority of polypeptides are synthesised within the cytosol from nuclear gene transcripts. Respiratory chain complexes I, III, IV, and the ATP synthase (complex V) contain both nuclear and mitochondrial subunits, but complex II has a purely nuclear origin. This means that patients with a pure complex II defect must have a nuclear gene defect,[83] but patients who have single complex defects that affect I, III, or IV may have a gene defect in either the mitochondrial or the nuclear genome. Adults with mitochondrial disease often have multiple deficiencies in respiratory chain complexes. This finding suggests a more global defect in mitochondrial biogenesis, which is often the result of a mutation involving a mitochondrial transfer RNA gene (fig 1). Further evidence of mtDNA tRNA defect can be gleaned from the histochemistry. Because the level of mutant mtDNA varies from cell to cell (see above), it is often possible to see a mosaic pattern of activity in skeletal muscle, with some fibres positive and some fibres negative for cytochrome c oxidase (COX, complex IV) in these patients.

Southern blotting of genomic DNA, extracted from skeletal muscle, with a range of restriction enzymes will identify duplications and deletions of mtDNA. Common point mutations should be screened by PCR/RFLP followed, as indicated, by direct sequencing of the entire mitochondrial genome. If a heteroplasmic rearrangement, or a recognised pathogenic point mutation is identified, molecular genetic diagnosis is relatively straightforward. Of the 150 patients who we have investigated for possible mitochondrial disease over the last 10 years, a positive finding has been possible in 46% of these cases. However, investigation of the large remainder remains a challenge, and how one should investigate these cases will be the focus of a second article.

## Management of patients with mtDNA disease

Although we cannot cure patients with mtDNA disease, optimal management can have a major impact on the level of morbidity and premature death. In broad terms, current management can be divided into three groups: (1) counselling, (2) supportive therapy, and (3) pharmacological therapy. We will then discuss some of the novel treatment strategies which are currently under development and provide some hope for the future.

## What does a mtDNA mutation mean for a person?

PROGNOSIS

Clinical and genetic variability are the hallmarks of mtDNA disease. For example, although the A3243G MELAS mutation is found in asymptomatic relatives, it can cause a mild phenotype, such as sensorineural deafness with diabetes, or alternatively it can cause a severe encephalopathy with death at a young age. Because the level of mutant mtDNA determines whether the genetic defect is expressed at the level of the cell, it seems axiomatic that there should be a simple relationship between mutation load and clinical disease. It is only recently, however, that there have been sufficient cumulative data to permit analysis. In a recent study of over 150 patients with the A3243G (MELAS) and A8344G (MERRF) mutations, the level of mutant mtDNA in skeletal muscle was highly correlated with the frequency of the major neurological features associated with each mutation.[109] In contrast, however, the clinical features were not related to the level of mutant mtDNA in blood. It appears that, in these patients, high levels of mutant mtDNA are found in postmitotic (non-dividing) tissues such as muscle and neurones, whereas lower levels are found in rapidly dividing tissues such as blood (which remains unaffected clinically). Thus, at least for MELAS and MERRF, the level of mutant mtDNA in a patient's muscle may give a reasonable guide to prognosis. However, any prognostic advice must be given with caution because the level of mutant mtDNA in muscle may increase[65] or decrease[170] with time.

PRESYMPTOMATIC TESTING

Over the last few years, we have analysed a number of extended pedigrees that transmit pathogenic mtDNA mutations. As one consequence of these studies, a number of unaffected adult relatives have sought advice about their own prognosis. Because of potential longitudinal changes of mutation load over time, this is a difficult issue. Nonetheless, the available data suggest that, at least for the A3243G and A8344G mutations, the level of mutant mtDNA in muscle may provide a guide to the future. This information must be given with extreme caution, because nuclear genetic and environmental factors may influence the expression of a mtDNA defect.[171 172] For these reasons, it is sensible to interpret the molecular genetic data within the context of the family being studied, modifying the estimated risks on the basis of the clinical outcome in other family members and the muscle mutation load of other family members.

INHERITANCE

Although mtDNA deletions themselves are not transmitted,[173 174] mtDNA duplications may be transmitted from mothers to offspring.[175–178] The duplications themselves are not pathogenic,[179] but they predispose to deletion formation.[180] It should be stressed that mtDNA duplications are rare, but a female harbouring a mtDNA duplication may thus have an affected child who also harbours a pathogenic mtDNA deletion.

MtDNA point mutations are also inherited down the maternal line.[93] For the homoplasmic mutations associated with LHON, extensive pedigree analyses have characterised the risks of blindness in maternal relatives.[181 182] However, for heteroplasmic mtDNA point mutations such as those that cause MELAS and MERRF, the situation is more complex. For these diseases, the outcome of pregnancy is dependent both upon the amount of mutant mtDNA transmitted to the oocyte and the segregation of mutant genomes during devel-

opment. On average, one would expect mothers with high levels of mutant mtDNA to transmit high levels of mutant mtDNA to their offspring. However, analysis of the transmission of the heteroplasmic mtDNA mutations has shown that a simple correlation is not obtained, and that there is a high variability in the mutation load among offspring. A major contribution to this high variability in mtDNA transmission is a restriction/amplification event (called a "bottleneck" by some authors) that occurs during oogenesis.[93] Although there have been recent advances in our understanding of the "bottleneck",[183] it is unlikely that the resulting simple mathematical models will be of any practical use in the clinic.[184] In the first place, the theoretical "bottleneck" size is calculated from the level of mutant mtDNA in the blood of a mother and her offspring. For the reasons outlined earlier, the level of mutant mtDNA in blood may change with time, and therefore it may not directly reflect the level of mutant mtDNA in the oocyte. Variation in the level of mutant mtDNA in blood may give the impression of an excessively tight "bottleneck". Secondly, bottleneck models predict that the range of possible outcomes of a pregnancy is huge. For any female, she would be predicted to have offspring harbouring either very low or very high levels of mutant mtDNA.

It is clear that we have a great deal to learn about the mechanism of inheritance of mtDNA mutations and their segregation. Although the "bottleneck" hypothesis may describe the events occurring in vivo, the fine details need to be determined and reliable genetic counselling will only be possible when we have a much more complete understanding of the processes involved in the inheritance, segregation, and expression of mtDNA defects. It was precisely because of these uncertainties that we took a different approach to the problem. Without making any assumptions about mechanism, we studied a large group of mothers who harbour the A3243G MELAS and A8344G MERRF mutations.[185] It was found that higher levels of mutant mtDNA in the mother's blood were associated with an increased frequency of affected offspring, but that the inheritance pattern of the two mutations was different. Although it would currently be unwise to use these data for genetic counselling, the results clearly indicate that the level of maternal mutant mtDNA is important in determining the frequency of clinically affected offspring. With the acquisition of prospective data, it should be possible to improve the genetic advice we give to females in the future.

PRENATAL DIAGNOSIS
The complexities of mtDNA segregation cause difficulties with prenatal diagnosis. For example, the level of mutant mtDNA in a chorionic villus sample (CVS) may be different from the level in the fetus. In isolated cases, the level of mutant mtDNA has been uniformly distributed throughout the fetus, which suggests that a chorionic villus sample might give useful information.[186] [187] However, at present we know little about how the level of mutant mtDNA might change during development, and it will be some time before we know whether the mutation load in the CVS provides clinically useful information.

As far as mtDNA point mutations are concerned, in our experience, mutant mtDNA can be detected in at least some tissues of almost every offspring of a female who harbours a pathogenic mutation, and CVS is thus likely to be positive for all pregnancies of an affected female. At present, we do not know the long term implications of a CVS heteroplasmy measurement, and it would be unwise to counsel purely on this basis. Further studies must be done in this area, but there are considerable ethical difficulties with a procedure that is not without risk.

PHARMACOLOGICAL THERAPY
At present, we have no effective disease modifying treatment for mtDNA disease.[188] Various vitamins and cofactors have been used, and varying degrees of success have been reported in individual cases.[189–191] The small clinical trials that have been carried out have not shown a consistent clinical effect,[192] but, because drugs such as ubiquinone (CoQ10) are relatively free of side effects, there is a rationale for their use in individual cases. In our experience, many patients report a modest improvement, but this is not sustained, suggesting a substantial placebo component to their response.

NOVEL TREATMENT STRATEGIES
Previous attempts at drug or metabolic therapy for mitochondrial diseases have not been encouraging. A moderate degree of exercise has been shown to improve exercise tolerance and muscle metabolism, and this should be recommended to patients with mtDNA disease,[193] and some recent laboratory research suggests that real progress may be possible in the near future. Recent in vitro work has shown that sequence specific peptide nucleic acids can inhibit the replication of mutant mtDNA.[194] These novel antigenomic molecules can be delivered into human mitochondria in culture, and potentially they may correct a mitochondrial genetic defect in vivo. Secondly, muscle satellite cells are the quiescent precursors of mature skeletal muscle and they contain low levels of mutant mtDNA. The induction of focal muscle cell necrosis induces satellite cell proliferation. In two patients with a mitochondrial myopathy, the subsequent proliferation of satellite cells led to the repopulation of a small region of skeletal muscle with mature muscle that harboured low levels of mutant mtDNA and with normal mitochondrial function.[195] [196]

**Conclusion**
In this review, we have outlined the common clinical presentations of mtDNA disease, highlighting the importance of a structured, integrated approach to the investigation of these patients. We have discussed what the diagnosis means for the person and described some of the difficulties in giving prognostic advice and genetic counselling. Over the last five years,

there has been a vast increase in our understanding of mtDNA disease. This progress has been paralleled by some advances in the clinical management of these patients. However, by the time we come to investigate patients with mtDNA disease, neuronal loss is often extensive and it is unlikely that we will be in a position to reverse the damage that has already been done. In addition, because the level of mutant mtDNA may change with time, it is crucial that we come to understand the mechanisms leading up to the clinical presentation. Although a contentious issue, it is therefore essential for us to study these people before they develop symptoms and signs. In this way, we will eventually be able to give more accurate prognostic information to subjects looking for a presymptomatic diagnosis, and it will be particularly important if we are going to develop disease modifying therapies which prevent the onset of neuronal loss.

1 Koutnikova H, Campuzano V, Foury F, Dolle P, Cazzalini O, Koenig M. Studies of human, mouse and yeast homologues indicate a mitochondrial function for frataxin. *Nat Genet* 1997;**16**:345-51.
2 Rotig A, de Lonlay P, Chretien D, *et al*. Aconitase and mitochondrial iron-sulphur protein deficiency in Friedreich ataxia. *Nat Genet* 1997;**17**:215-17.
3 Lutsenko S, Cooper MJ. Localization of the Wilson's disease protein product to mitochondria. *Proc Natl Acad Sci USA* 1998;**95**:6004-9.
4 Casari G, De Fusco M, Ciarmatori S, *et al*. Spastic paraplegia and OXPHOS impairment caused by mutations in paraplegin, a nuclear-encoded mitochondrial metalloprotease. *Cell* 1998;**93**:973-83.
5 Holt I, Harding AE, Morgan-Hughes JA. Deletion of muscle mitochondrial DNA in patients with mitochondrial myopathies. *Nature* 1988;**331**:717-19.
6 Moraes CT, DiMauro S, Zeviani M, *et al*. Mitochondrial DNA deletions in progressive external ophthalmoplegia and Kearns-Sayre syndrome. *N Engl J Med* 1989;**320**:1293-9.
7 Zeviani M, Moraes CT, DiMauro S, *et al*. Deletions of mitochondrial DNA in Kearns-Sayre syndrome. *Neurology* 1988;**38**:1339-46.
8 Cormier V, Rotig A, Rasore Q, *et al*. Widespread multitissue deletions of the mitochondrial genome in Pearsons marrow-pancreas syndrome. *J Pediatr* 1989;**117**:599-602.
9 Ballinger SW, Shoffner JM, Hedaya EV, *et al*. Maternally transmitted diabetes and deafness associated with a 10.4 kb mitochondrial DNA deletion. *Nat Genet* 1992;**1**:11-15.
10 Chalmers RM, Brockington M, Howard RS, Lecky BR, Morgan-Hughes JA, Harding AE. Mitochondrial encephalopathy with multiple mitochondrial DNA deletions. *J Neurol Sci* 1996;**143**:41-5.
11 Ohno K, Tanaka M, Sahashi K. Mitochondrial DNA deletions in inherited recurrent myoglobinuria. *Ann Neurol* 1991;**29**:364-9.
12 Fadic R, Russell JA, Vedanarayanan VV, Lehar M, Kuncl RW, Johns DR. Sensory ataxic neuropathy as the presenting feature of a novel mitochondrial disease. *Neurology* 1997;**49**:239-45.
13 Wallace DC, Singh G, Lott MT, *et al*. Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. *Science* 1988;**242**:1427-30.
14 Howell N, Kubacka I, Xu M, McCullough DA. Leber hereditary optic neuropathy: involvement of the mitochondrial ND1 gene and evidence for an intragenic suppressor mutation. *Am J Hum Genet* 1991;**48**:935-42.
15 Johns DR, Neufeld MJ, Park RD. An ND-6 mitochondrial DNA mutation associated with Leber hereditary optic neuropathy. *Biochem Biophys Res Commun* 1992;**187**:1551-7.
16 De Vries DD, Went LN, Bruyn GW, *et al*. Genetic and biochemical impairment of mitochondrial complex I activity in a family with Leber hereditary optic neuropathy and hereditary spastic dystonia. *Am J Hum Genet* 1996;**58**:703-11.
17 Jun AS, Brown MD, Wallace DC. A mitochondrial DNA mutation at nucleotide pair 14459 of the NADH dehydrogenase subunit 6 gene associated with maternally inherited Leber hereditary optic neuropathy and dystonia. *Proc Natl Acad Sci USA* 1994;**91**:6206-10.
18 Manfredi G, Schon EA, Moraes CT, *et al*. A new mutation associated with MELAS is located in a mitochondrial DNA polypeptide-coding gene. *Neuromusc Disord* 1995;**5**:391-8.
19 Santorelli FM, Tanji K, Kulikova R, *et al*. Identification of a novel mutation in the mtDNA ND5 gene associated with MELAS. *Biochem Biophys Res Commun* 1997;**238**:326-8.
20 Hanna MG, Nelson IP, Rahman S, *et al*. Cytochrome c oxidase deficiency associated with the first stop-codon point mutation in human mtDNA. *Am J Hum Genet* 1998;**63**:29-36.
21 Campos Y, Martin MA, Rubio JC, Gutierrez del Olmo MC, Cabello A, Arenas J. Bilateral striatal necrosis and MELAS associated with a new T3308C mutation in the mitochondrial ND1 gene. *Biochem Biophys Res Commun* 1997;**238**:323-5.
22 De Meirleir L, Seneca S, Lissens W, Schoentjes E, Desprechins B. Bilateral striatal necrosis with a novel point mutation in the mitochondrial ATPase 6 gene. *Pediatr Neurol* 1995;**13**:242-6.
23 Holt IJ, Harding AE, Petty RK, Morgan-Hughes JA. A new mitochondrial disease associated with mitochondrial DNA heteroplasmy. *Am J Hum Genet* 1990;**46**:428-33.
24 de Vries DD, van Engelen BGM, Gabreels FJM, Ruitenbeek W, van Oost BA. A second missense mutation in the mitochondrial ATPase 6 gene mutation in Leigh's syndrome. *Ann Neurol* 1993;**34**:410-12.
25 Campos Y, Martin MA, Rubio JC, *et al*. Leigh syndrome associated with the T9176C mutation in the ATPase 6 gene of mitochondrial DNA. *Neurology* 1997;**49**:595-7.
26 Thyagarajan D, Shanske S, Vazquez-Memije M, De Vivo D, DiMauro S. A novel mitochondrial ATPase 6 point mutation in familial bilateral striatal necrosis. *Ann Neurol* 1995;**38**:468-72.
27 Taylor RW, Chinnery PF, Haldane F, *et al*. MELAS associated with a mutation in the valine transfer RNA gene of mitochondrial DNA. *Ann Neurol* 1996;**40**:459-62.
28 Goto Y, Nonaka I, Horai S. A mutation in the tRNA(Leu)(UUR) gene associated with the MELAS subgroup of mitochondrial encephalomyopathies. *Nature* 1990;**348**:651-3.
29 Morten KJ, Cooper JM, Brown GK, Lake BD, Pike D, Poulton J. A new point mutation associated with mitochondrial encephalomyopathy. *Hum Mol Genet* 1993;**2**:2081-7.
30 Nishino I, Komatsu M, Kodama S, Horai S, Nonaka I, Goto Y. The 3260 mutation in mitochondrial DNA can cause mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes (MELAS). *Muscle Nerve* 1996;**19**:1603-4.
31 Goto YI, Nonaka I, Horai S. A new mtDNA mutation associated with mitochondrial myopathy, encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS). *Biochem Biophys Acta* 1991;**1097**:238-40.
32 Goto Y, Tsugane K, Tanabe Y, Nonaka I, Horai S. A new point mutation at nucleotide pair 3291 of the mitochondrial tRNA(Leu(UUR)) gene in a patient with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS). *Biochem Biophys Res Commun* 1994;**202**:1624-30.
33 Manfredi G, Schon EA, Bonilla E, Moraes CT, Shanske S, DiMauro S. Identification of a mutation in the mitochondrial tRNA(Cys) gene associated with mitochondrial encephalopathy. *Hum Mutat* 1996;**7**:158-63.
34 Shoffner JM, Lott MT, Lezza AM, Seibel P, Ballinger SW, Wallace DC. Myoclonic epilepsy and ragged-red fiber disease (MERRF) is associated with a mitochondrial DNA tRNA(Lys) mutation. *Cell* 1990;**61**:931-7.
35 Silvestri G, Moraes CT, Shanske S, Oh SJ, DiMauro S. A new mtDNA mutation in the tRNA(Lys) gene associated with myoclonic epilepsy and ragged-red fibers (MERRF). *Am J Hum Genet* 1992;**51**:1213-7.
36 Ozawa M, Nishino I, Horai S, Nonaka I, Goto YI. Myoclonus epilepsy associated with ragged-red fibers: a G-to-A mutation at nucleotide pair 8363 in mitochondrial tRNA(Lys) in two families. *Muscle Nerve* 1997;**20**:271-8.
37 Nakamura M, Nakano S, Goto Y, *et al*. A novel point mutation in the mitochondrial tRNA(Ser(UCN)) gene detected in a family with MERRF/MELAS overlap syndrome. *Biochem Biophys Res Commun* 1995;**214**:86-93.
38 Zeviani M, Muntoni F, Savarese N, *et al*. A MERRF/MELAS overlap syndrome associated with a new point mutation in the mitochondrial DNA tRNA(Lys) gene (published erratum appears in *Eur J Hum Genet* 1993;**1**:124). *Eur J Hum Genet* 1993;**1**:80-7.
39 Reardon W, Ross RJ, Sweeney MG, *et al*. Diabetes mellitus associated with a pathogenic point mutation in mitochondrial DNA. *Lancet* 1992;**340**:1376-9.
40 Lynn S, Wardell T, Johnson MA, *et al*. Mitochondrial diabetes: investigation and identification of a novel mutation. *Diabetes* 1998;**47**:1800-2.
41 Silvestri G, Bertini E, Servidei S, *et al*. Maternally inherited cardiomyopathy: a new phenotype associated with the A to G AT nt.3243 of mitochondrial DNA (MELAS mutation). *Muscle Nerve* 1997;**20**:221-5.
42 Kawarai T, Kawakami H, Kozuka K, *et al*. A new mitochondrial DNA mutation associated with mitochondrial myopathy: tRNA(Leu)(UUR) 3254C-to-G. *Neurology* 1997;**49**:598-600.
43 Zeviani M, Gellera C, Antozzi C, *et al*. Maternally inherited myopathy and cardiomyopathy: association with mutation in mitochondrial DNA tRNA Leu(UUR). *Lancet* 1991;**338**:143-7.

44 Silvestri G, Santorelli FM, Shanske S, *et al*. A new mtDNA mutation in the tRNA(Leu(UUR)) gene associated with maternally inherited cardiomyopathy. *Hum Mutat* 1994;**3**: 37–43.

45 Taniike M, Fukushima H, Yanagihara I, *et al*. Mitochondrial tRNA$^{Ile}$ mutation in fatal cardiomyopathy. *Biochem Biophys Res Commun* 1992;**186**:47–53.

46 Merante F, Myint T, Tein I, Benson L, Robinson BH. An additional mitochondrial tRNA(Ile) point mutation (A-to-G at nucleotide 4295) causing hypertrophic cardiomyopathy. *Hum Mutat* 1996;**8**:216–22.

47 Casali C, Santorelli FM, Gu DA, Bernucci P, DeBiase L, DiMauro S. A novel mtDNA point mutation in maternally inherited cardiomyopathy. *Biochem Biophys Res Commun* 1995;**213**:588–93.

48 Santorelli FM, Mak SC, Vazquez-Acevedo M, *et al*. A novel mitochondrial DNA point mutation associated with mitochondrial encephalocardiomyopathy. *Biochem Biophys Res Commun* 1995;**216**:835–40.

49 Santorelli FM, Mak SC, El-Schahawi M, *et al*. Maternally inherited cardiomyopathy and hearing loss associated with a novel mutation in the mitochondrial tRNA(Lys) gene (G8363A). *Am J Hum Genet* 1996;**58**:933–9.

50 Merante F, Tein I, Benson L, Robinson BH. Maternally inherited hypertrophic cardiomyopathy due to a novel T-to-C transition at nucleotide 9997 in the mitochondrial tRNA(glycine) gene. *Am J Hum Genet* 1994;**55**:437–46.

51 Moraes CT, Ciacci F, Silvestri G, *et al*. Atypical clinical presentations associated with the MELAS mutation at position 3243 of human mitochondrial DNA. *Neuromusc Disord* 1993;**3**:43–50.

52 Sweeney MG, Bundey S, Brockington M, Poulton KR, Winer JB, Harding AE. Mitochondrial myopathy associated with sudden death in young adults and a novel mutation in the mitochondrial DNA leucine transfer RNA(UUR) gene. *Q J Med* 1993;**86**:709–13.

53 Moraes CT, Ciacci F, Bonilla E, *et al*. Two novel pathogenic mitochondrial DNA mutations affecting organelle number and protein synthesis. Is the tRNA(Leu(UUR) gene an etiologic hot spot? *J Clin Invest* 1993;**92**:2906–15.

54 Chinnery PF, Johnson MA, Taylor RW, Durward WF, Turnbull DM. A novel mitochondrial tRNA isoleucine gene mutation causing chronic progressive external ophthalmoplegia. *Neurology* 1997;**49**:1166–8.

55 Silvestri G, Servidei S, Rana M, *et al*. A novel mitochondrial DNA point mutation in the tRNA(Ile) gene is associated with progressive external ophthalmoplegia. *Biochem Biophys Res Commun* 1996;**220**:623–7.

56 Taylor RW, Chinnery PF, Bates MJD, *et al*. A novel mitochondrial DNA point mutation in the tRNA$^{Ile}$ gene: studies in a patient presenting with chronic progressive external ophthalmoplegia and multiple sclerosis. *Biochem Biophys Res Commun* 1998;**243**:47–51.

57 Seibel P, Lauber J, Klopstock T, Marsac C, Kadenbach B, Reichmann H. Chronic progressive external ophthalmoplegia is associated with a novel mutation in the mitochondrial tRNA(Asn) gene. *Biochem Biophys Res Commun* 1994; **204**:482–9.

58 Hattori Y, Goto YI, Sakuta R, Nonaka I, Mizuno Y, Horai S. Point mutations in mitochondrial tRNA genes: sequence analysis of chronic progressive external ophthalmoplegia (CPEO). *J Neurol Sci* 1994;**125**:50–5.

59 Fu K, Hartien R, Johns T, Genge A, Karpati G, Shoubridge EA. A novel heteroplasmic tRNA$^{leu(UUR)}$ mtDNA point mutation in a sporadic patient with mitochondrial encephalomyopathy segregates rapidly in muscle and suggests an approach to therapy. *Hum Mol Genet* 1996;**5**:1835–40.

60 Chinnery PF, Johnson MA, Taylor RW, Lightowlers RN, Turnbull DM. A novel mitochondrial tRNA phenylalanine mutation presenting with acute rhabdomyolysis. *Ann Neurol* 1997;**41**:408–10.

61 Goto Y, Tojo M, Tohyama J, Horai S, Nonaka I. A novel point mutation in the mitochondrial tRNA(Leu)(UUR) gene in a family with mitochondrial myopathy. *Ann Neurol* 1992;**31**:672–5.

62 Bindoff LA, Howell N, Poulton J, *et al*. Abnormal RNA processing associated with a novel tRNA mutation in mitochondrial DNA. A potential disease mechanism. *J Biol Chem* 1993;**268**:19559–64.

63 Vissing J, Salamon MB, Arlein-Soborg P, *et al*. A new mitochondrial tRNA$^{Met}$ gene mutation in a patient with dystrophic muscle and excercise intolerance. *Neurology* 1998;**50**:1875–8.

64 Silvestri G, Rana M, DiMuzio A, Uncini A, Tonali P, Servidei S. A late-onset mitochondrial myopathy is associated with a novel mitochondrial DNA (mtDNA) point mutation in the tRNA$^{Trp}$ gene. *Neuromusc Disord* 1998;**8**:291–5.

65 Weber K, Wilson JN, Taylor L, *et al*. A new mtDNA mutation showing accumulation with time and restriction to skeletal muscle. *Am J Hum Genet* 1997;**60**:373–80.

66 Moraes CT, Ciacci F, Bonilla E, Ionasescu V, Schon EA, DiMauro S. A mitochondrial tRNA anticodon swap associated with a muscle disease. *Nat Genet* 1993;**4**:284–8.

67 Hanna MG, Nelson I, Sweeney MG, *et al*. Congenital encephalomyopathy and adult-onset myopathy and diabetes mellitus: different phenotypic associations of a new heteroplasmic mtDNA tRNA glutamic acid mutation. *Am J Hum Genet* 1995;**56**:1026–33.

68 Tiranti V, D'Agruma L, Pareyson D, *et al*. A novel mutation in the mitochondrial tRNA(Val) gene associated with a complex neurological presentation. *Ann Neurol* 1998;**43**: 98–101.

69 Bidooki SK, Johnson MA, Chrzanowska-Lightowlers Z, Bindoff LA, Lightowlers RN. Intracellular mitochondrial triplasmy in a patient with two, heteroplasmic base changes. *Am J Hum Genet* 1997;**60**:1430–8.

70 Santorelli FM, Tanji K, Sano M, *et al*. Maternally inherited encephalopathy associated with a single-base insertion in the mitochondrial tRNATrp gene. *Ann Neurol* 1997;**42**: 256–60.

71 Nelson I, Hanna MG, Alsanjari N, Scaravilli F, Morgan-Hughes JA, Harding AE. A new mitochondrial DNA mutation associated with progressive dementia and chorea: a clinical, pathological, and molecular genetic study. *Ann Neurol* 1995;**37**:400–3.

72 Chinnery PF, Turnbull DM. Vomiting, anorexia and mitochondrial DNA disease. *Lancet* 1998;**351**:448.

73 Verma A, Piccoli DA, Bonilla E, Berry GT, DiMauro S, Moraes CT. A novel mitochondrial G8313A mutation associated with prominent initial gastrointestinal symptoms and progressive encephaloneuropathy. *Pediatr Res* 1997;**42**:448–54.

74 Chalmers RM, Lamont PJ, Nelson I, *et al*. A mitochondrial DNA tRNA(Val) point mutation associated with adult-onset Leigh syndrome. *Neurology* 1997;**49**:589–92.

75 Reid FM, Vernham GA, Jacobs HT. A novel mitochondrial point mutation in a maternal pedigree with sensorineural deafness. *Hum Mutat* 1994;**3**:243–7.

76 Gattermann N, Retzlaff S, Wang YL, *et al*. A heteroplasmic point mutation of mitochondrial tRNALeu(CUN) in non-lymphoid haemopoietic cell lineages from a patient with acquired idiopathic sideroblastic anaemia. *Br J Haematol* 1996;**93**:845–55.

77 Prezant TR, Agapian JV, Bohlman MC, *et al*. Mitochondrial ribosomal RNA mutations associated with both antibiotic-induced and non-sydromic deafness. *Nat Genet* 1993;**4**: 289–94.

78 Suomalainen A, Kaukonen J, Amati P, *et al*. An autosomal locus predisposing to deletions of mitochondrial DNA. *Nat Genet* 1995;**9**:146–51.

79 Kaukonen JA, Amati P, Suomalainen A, *et al*. An autosomal locus predisposing to multiple deletions of mtDNA on chromosome 3p. *Am J Hum Genet* 1996;**58**:763–9.

80 Nishino I, Spinazzola A, Hirano M. Thymidine phosphorylase gene mutations in MNGIE, a human mitochondrial disorder. *Science* 1999;**283**:689–92.

81 Van den Heuvel L, Ruitenbeek W, Smeets R, *et al*. Demonstration of a new pathogenic mutation in human complex I deficiency: a 5-bp duplication in the nuclear gene encoding the 18kD (AQDQ) subunit. *Am J Hum Genet* 1998;**62**:262–8.

82 Loeffen J, Smeitink J, Treipels R, *et al*. The first nuclear-encoded complex I mutation in a patient with Leigh syndrome. *Am J Hum Genet* 1998;**63**:1598–608.

83 Taylor RW, Birch-Machin MA, Schaefer J, *et al*. Deficiency of complex II of the mitochondrial respiratory chain in late-onset optic atrophy and ataxia. *Ann Neurol* 1996;**39**: 224–32.

84 Bougeron T, Rustin P, Birch-Machin M, *et al*. A mutation of nuclear succinate dehydrogenase gene results in mitochondrial respiratory chain deficiency. *Nat Genet* 1995;**11**:144–9.

85 Zhu Z, Yao J, Johns T, *et al*. SURF1, encoding a factor involved in the biogenesis of cytochrome c oxidase, is mutated in Leigh syndrome. *Nat Genet* 1998;**20**:337–43.

86 Tiranti V, Hoertnagel K, Carrozzo R, *et al*. Mutations of SURF-1 in Leigh disease associated with cytochrome c oxidase deficiency. *Am J Hum Genet* 1998;**63**:1609–21.

87 Tritschler HJ, Andreetta F, Moraes CT, *et al*. Mitochondrial myopathy of childhood associated with depletion of mitochondrial DNA. *Neurology* 1992;**42**:209–17.

88 Moraes CT, Shanske S, Tritschler HJ, *et al*. mtDNA depletion with variable tissue expression: a novel genetic abnormality in mitochondrial diseases. *Am J Hum Genet* 1991;**48**:492–501.

89 Anderson S, Bankier AT, Barrell BG, *et al*. Sequence and organization of the human mitochondrial genome. *Nature* 1981;**290**:457–65.

90 Chomyn A, Meola G, Bresolin N, Lai ST, Scarlato G, Attardi G. In vitro genetic transfer of protein synthesis and respiration defects to mitochondrial DNA-less cells with myopathy-patient mitochondria. *Mol Cell Biol* 1991;**11**: 2236–44.

91 Enriquez JA, Chomyn A, Attardi G. MtDNA mutation in MERRF syndrome causes defective aminoacylation of tRNA(Lys) and premature translation termination. *Nat Genet* 1995;**10**:47–55.

92 Howell N. Human mitochondrial diseases: answering questions and questioning answers. *Int Rev Cytol* 1999;**186**:49–116.

93 Lightowlers RN, Chinnery PF, Turnbull DM, Howell N. Mammalian mitochondrial genetics: heredity, heteroplasmy and disease. *Trends Genet* 1997;**13**:450–5.

94 Schon EA, Bonilla E, DiMauro S. Mitochondrial DNA mutations and pathogenesis. *J Bioenerg Biomembr* 1997;**29**: 131–49.

95 Servidei S. Mitochondrial encephalomyopathies: gene mutation. *Neuromusc Disord* 1999;**9**:XV-XXII.

96 Chinnery PF, Turnbull DM. Mitochondrial medicine. *Q J Med* 1997;**90**:657–66.

97 Chinnery PF, Turnbull DM. The clinical features, investigation and management of patients with mitochondrial DNA defects. *J Neurol Neurosurg Psychiatry* 1997;**63**:559–63.

98 Hirano M, Ricci E, Koenigsberger MR, *et al*. Melas: an original case and clinical criteria for diagnosis. *Neuromusc Disord* 1992;**2**:125–35.

99 Gerbitz K-D, van den Ouweland JMW, Maasen JA, Jaksch M. Mitochondrial diabetes: a review. *Biochem Biophys Acta* 1995;**1271**:253-60.

100 Vilarinho L, Santorelli FM, Rosas MJ, Tavares C, Melo-Pires M, DiMauro S. The mitochondrial A3243G mutation presenting as severe cardiomyopathy. *J Med Genet* 1997;**34**:607-9.

101 Nikoskelainen EK. Clinical picture of LHON. *Clin Neurosci* 1994;**2**:115-20.

102 Hanna MG, Vaughan JR, Silburn PA, *et al*. Two unusual clinical presentations of the mitochondrial DNA A3243G point mutation in adult neurological practice. *J Neurol Neurosurg Psychiatry* 1997;**62**:544-5.

103 Kimata KG, Gordan L, Ajax ET, Davis PH, Grabowski T. A case of late onset MELAS. *Arch Neurol* 1998;**55**:722-5.

104 Howell N. Leber hereditary optic neuropathy: how do mitochondrial DNA mutations cause degeneration of the optic nerve? *J Bioenerg Biomembr* 1997;**29**:165-73.

105 Estivill X, Govea N, Barcelo A, *et al*. Familial progressive sensorineural deafness is mainly due to the mtDNA A1555G mutation and is enhanced by treatment with aminoglycosides. *Am J Hum Genet* 1997;**62**:27-35.

106 Sue CM, Lipsett LJ, Crimmins DS, *et al*. Cochlear origin of hearing loss in MELAS syndrome. *Ann Neurol* 1998;**43**: 350-9.

107 Mosewich RK, Donat JR, DiMauro S, *et al*. The syndrome of mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes presenting without stroke. *Arch Neurol* 1993;**50**:275-8.

108 Klopstock T, May A, Seibel P, Papagiannuli E, Diener HC, Reichmann H. Mitochondrial DNA in migraine with aura. *Neurology* 1996;**46**:1735-8.

109 Chinnery P, Howell N, Lightowlers R, Turnbull D. Molecular pathology of MELAS and MERRF: the relationship between mutation load and clinical phenotype. *Brain* 1997;**120**:1713-21.

110 Wallace DC, Lott MT, Shoffner JM, Ballinger S. Mitochondrial DNA mutations in epilepsy and neurological disease. *Epilepsia* 1994;**35**(suppl 1):S43-50.

111 Jackson MJ, Schaefer JA, Johnson MA, Morris AAM, Turnbull DM, Bindoff LA. Presentation and clinical investigation of mitochondrial respiratory chain disease. *Brain* 1995;**118**:339-57.

112 Shoffner JM, Kaufman A, Koontz D, *et al*. Oxidative phosphorylation diseases and cerebellar ataxia. *Clin Neurosci* 1995;**3**:43-53.

113 Shoffner JM, Wallace DC. Oxidative phosphorylation diseases and stroke. *Heart Dis Stroke* 1993;**2**:439-45.

114 Hanna MG, Bhatia KP. Movement disorders and mitochondrial dysfunction. *Curr Opin Neurol* 1997;**10**:351-6.

115 Hirano M, DiMauro S. Clinical features of mitochondrial myopathies and encephalomyopathies. In: Lane RJ, ed. *Handbook of muscle disease*. New York: Marcel Dekker, 1996:479-504.

116 Chu CC, Huang CC, Fang W, Chu NS, Pang CY, Wei YH. Peripheral neuropathy in mitochondrial encephalomyopathies. *Eur Neurol* 1997;**37**:110-15.

117 Hammans SR, Sweeney MG, Hanna MG, Brockington M, Morgan-Hughes JA, Harding AE. The mitochondrial DNA transfer RNALeu(UUR) A→G(3243) mutation. A clinical and genetic study. *Brain* 1995;**118**(pt 3):721-34.

118 Harding AE, Riordan-Eva P, Govan GG. Mitochondrial DNA diseases: genotype and phenotype in Leber's hereditary optic neuropathy. *Muscle Nerve* 1995;**3**:S82-4.

119 Zeviani M, Mariotti C, Antozzi C, Fratta GM, Rustin P, Prelle A. OXPHOS defects and mitochondrial DNA mutations in cardiomyopathy. *Muscle Nerve* 1995;**3**:S170-4.

120 Gerbitz KD, Gempel K, Brdiczka D. Mitochondria and diabetes. Genetic, biochemical, and clinical implications of the cellular energy circuit. *Diabetes* 1996;**45**:113-26.

121 Papadimitiou A, Hadjigeorgiou GM, Divari R, Papagalanis N, Comi G, Bresolin N. Influence of coenzyme Q10 on total serum calcium concentration in two patients with Kearns-Sayre syndrome and hypoparathyroidism. *Neuromusc Disord* 1996;**6**:49-53.

122 Tulinius MH, Oldfors A, Holme E, *et al*. Atypical presentation of multisystem disorders in two girls with mitochondrial DNA deletions. *Eur J Pediatr* 1995;**154**:35-42.

123 Bhattacharyya A, Tymms DJ. Mitochondrial DNA defects and endocrine dysfunction. *Q J Med* 1998;**91**:375-6.

124 Chen CM, Huang CC. Gonadal dysfunction in mitochondrial encephalomyopathies. *Eur Neurol* 1995;**35**:281-6.

125 Folgero T, Bertheussen K, Lindal S, Torbergsen T, Oian P. Mitochondrial disease and reduced sperm motility. *Hum Reprod* 1993;**8**:1863-8.

126 Boles RG, Chun N, Senadheera D, Wond LJC. Cyclical vomiting syndrome and mitochondrial DNA mutations. *Lancet* 1997;**350**:1299-300.

127 Hirano M, Silvestri G, Blake DM, *et al*. Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE): clinical, biochemical, and genetic features of an autosomal recessive mitochondrial disorder. *Neurology* 1994;**44**:721-7.

128 Heil JAP, Verrips A, Keyser A, *et al*. Ileus in mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes. *Neth J Med* (in press).

129 Shoffner JM, Voljavec AS, Dixon J, Kaufman A, Wallace DC, Mitch WE. Renal amino acid transport in adults with oxidative phosphorylation diseases. *Kidney Int* 1995;**47**: 1101-7.

130 Eviatar L, Shanske S, Gauthier B, *et al*. Kearns-Sayre syndrome presenting as renal tubular acidosis. *Neurology* 1990;**40**:1761-3.

131 Szabolcs MJ, Seigle R, Shanske S, Bonilla E, DiMauro S. Mitochondrial DNA deletion: a cause of chronic tubulointerstitial nephropathy. *Kidney Int* 1994;**45**:1388-96.

132 Niaudet P, Heidet L, Munnich A, *et al*. Deletion of the mitochondrial DNA in a case of de Toni-Debre-Fanconi syndrome and Pearson syndrome. *Pediatr Nephrol* 1994;**8**: 164-8.

133 Morris AA, Taylor RW, Birch-Machin MA, *et al*. Neonatal Fanconi syndrome due to deficiency of complex III of the respiratory chain. *Pediatr Nephrol* 1995;**9**:407-11.

134 Goto Y, Itami N, Kajii N, Tochimaru H, Endo M, Horai S. Renal tubular involvement mimicking Bartter syndrome in a patient with Kearns-Sayre syndrome. *J Pediatr* 1990;**116**: 904-10.

135 Rotig A, Cormier V, Blanche S, Bonnefont JP, Ledeist F. Pearson's marrow pancreas syndrome. A multisystem mitochondrial disorder of infancy. *J Clin Invest* 1990;**86**: 1601-8.

136 Onishi H, Hanihara T, Sugiyama N, *et al*. Pancreatic exocrine dysfunction associated with mitochondrial tRNA<sup>Leu(UUR)</sup> mutation. *J Med Genet* 1998;**35**:255-7.

137 Munnich A, Rotig A, Chretien D, *et al*. Clinical presentation of mitochondrial disorders in childhood. *J Inherit Metab Dis* 1996;**19**:521-7.

138 Suomalainen A, Kaukonen J, Amati P, *et al*. An autosomal locus predisposing to deletions of mitochondrial DNA. *Nat Genet* 1995;**9**:146-51.

139 Onishi H, Kawanishi C, Iwasawa T, *et al*. Depressive disorder due to mitochondrial transfer RNALeu(UUR) mutation. *Biol Psychiatr* 1997;**41**:1137-9.

140 Thomeer EC, Verhoeven WMA, Van de Vlasakker CJW, Klompenhouwer JL. Psychiatric symptoms in MELAS: a case report. *J Neurol Neurosurg Psychiatry* 1998;**64**:692-3.

141 Larsson NG, Tulinius MH, Holme E, Oldfors A. Pathogenetic aspects of the A8344G mutation of mitochondrial DNA associated with MERRF syndrome and multiple symmetric lipomas. *Muscle Nerve* 1995;**3**:S102-6.

142 Howell N, Kubacka I, Smith R, Frerman F, Parks JK, Parker WD Jr. Association of the mitochondrial 8344 MERRF mutation with maternally inherited spinocerebellar degeneration and Leigh disease. *Neurology* 1996;**46**: 219-22.

143 Shoffner JM, Brown MD, Stugard C, *et al*. Leber's hereditary optic neuropathy plus dystonia is caused by a mitochondrial DNA point mutation. *Ann Neurol* 1995;**38**: 163-9.

144 Bruyn GW, Bots GT, Went LN, Klinkhamer PJ. Hereditary spastic dystonia with Leber's hereditary optic neuropathy: neuropathological findings. *J Neurol Sci* 1992; **113**:55-61.

145 Mansergh FC, Millington-Ward S, Kennan A, *et al*. Retinitis pigmentosa and progressive sensorineural hearing loss caused by a C12258A mutation in the mitochondrial MTTS2 gene. *Am J Hum Genet* 1999;**64**:971–85.

146 Henderson GV, Kittner SJ, Johns DR. An incidence study of stroke secondary to MELAS in the young. *Neurology* 1997;**48**:A403.

147 Majamma K, Turkka J, Karppa M, Winqvist S, Hassinen IE. The common MELAS mutation A3243G in mitochondrial DNA among patients with an occipital infarct. *Neurology* 1997;**49**:1331-4.

148 Merante F, Tein I, Benson L, Robinson BH. Maternally inherited hypertrophic cardiomyopathy due to a novel T-to-C transition at nucleotide 9997 in the mitochondrial tRNA<sup>glycine</sup> gene. *Am J Hum Genet* 1994;**55**:437-46.

149 Matsutani H, Mizusawa Y, Shimoda M, *et al*. Partial deficiency of cytochrome c oxidase with isolated proximal renal tubular acidosis and hypercalciuria. *Child Nephrol Urol* 1992;**12**:221-4.

150 Damian MS, Seibel P, Reichmann H, *et al*. Clinical spectrum of the MELAS mutation in a large pedigree. *Acta Neurol Scand* 1995;**92**:409-15.

151 Zupanc ML, Moraes CT, Shanske S, Langman CB, Ciafaloni E, DiMauro S. Deletion of mitochondrial DNA in patients with combined features of Kearns-Sayre and MELAS syndromes. *Ann Neurol* 1991;**29**:680-3.

152 North K, Korson MS, Krawiecki N, Shoffner JM, Holm IA. Oxidative phosphorylation defect associated with primary adrenal insufficiency. *J Pediatr* 1996;**128**(pt 1):688-92.

153 Bruno C, Minetti C, Tang Y, *et al*. Primary adrenal insufficiency in a child with a mitochondrial DNA deletion. *J Inherit Metab Dis* 1998;**21**:155-161.

154 Maassen J, Kadowaki T. Maternally inherited diabetes and deafness: a new diabetes subtype. *Diabetologia* 1996;**39**: 375-82.

155 Gardner JC, Goliath R, Viljoen D, *et al*. Familial streptomycin ototoxicity in a South African family: a mitochondrial disorder. *J Med Genet* 1997;**34**:904-6.

156 Brown GK, Otero LJ, LeGris M, Brown RM. Pyruvate dehydrogenase deficiency. *J Med Genet* 1994;**31**:875-9.

157 DiMauro S, DeVivo DC. Genetic heterogeneity in Leigh syndrome. *Ann Neurol* 1996;**40**:5-7.

158 Santorelli FM, Shanske S, Macaya A, DeVivo DC, DiMauro S. The mutation at nt 8993 of mitochondrial DNA is a common cause of Leigh's syndrome. *Ann Neurol* 1993;**34**:827-34.

159 Van der Knaap MS, Jakobs C, Valk J. Magnetic resonance imaging in lactic acidosis. *J Inherit Metab Dis* 1996;**19**:535-47.

160 Barkovich AJ, Good WV, Koch TK, Berg BO. Mitochondrial disorders: analysis of their clinical and imaging characteristics. *Am J Neuroradiol* 1993;**14**:1119-37.

161 Wray SH, Provenzale JM, Johns DR, Thulborn KR. MR of the brain in mitochondrial myopathy. *Am J Neuroradiol* 1995;**16**:1167-73.

162 Matthews PM, Tampieri D, Berkovic SF, *et al*. Magnetic resonance imaging shows specific abnormalities in the MELAS syndrome. *Neurology* 1991;**41**:1043-6.

163 Larsson NG, Clayton DA. Molecular genetic aspects of human mitochondrial disorders. *Annu Rev Genet* 1995;**29**:151-78.

164 Hammans SR, Sweeney MG, Brockington M, *et al*. The mitochondrial DNA transfer RNA(Lys)A→G(8344) mutation and the syndrome of myoclonic epilepsy with ragged red fibres (MERRF). Relationship of clinical phenotype to proportion of mutant mitochondrial DNA. *Brain* 1993;**116**(pt 3):617-32.

165 Howell N, Bindoff LA, McCullough DA, *et al*. Leber hereditary optic neuropathy: identification of the same mitochondrial ND1 mutation in six pedigrees. *Am J Hum Genet* 1991;**49**:939-50.

166 Howell N. Primary LHON mutations: trying to separate "fruyt" from "chaf". *Clin Neurosci* 1994;**2**:130-7.

167 Chinnery PF, Reading PJ, Walls TJ, Turnbull DM. Recurrent strokes in a 34 year old man. *Lancet* 1997;**350**:560.

168 Morgan-Hughes JA. Mitochondrial diseases. In: Engel AG, Franzini-Armstrong C, eds. *Myology*. New York: McGraw-Hill, 1994:1610-60.

169 Johnson MA, Barron MJ. Muscle biopsy analysis. In: Lane RJM, ed. *Handbook of muscle disease*. New York: Marcel Dekker, 1996.

170 Kawakami Y, Sakuta R, Hashimoto K, *et al*. Mitochondrial myopathy with progressive decrease in mitochondrial tRNA(Leu)(UUR) mutant genomes. *Ann Neurol* 1994;**35**:370-3.

171 Howell N. Leber hereditary optic neuropathy: mitochondrial mutations and degeneration of the optic nerve. *Vision Res* 1997;**37**:3495-507.

172 Chinnery PF, Zwijnenburg PJG, Howell N, *et al*. Non-random tissue distribution of mutant mitochondrial DNA. *Am J Med Genet* (in press).

173 Zeviani M, Gellera C, Pannacci M, *et al*. Tissue distribution and transmission of mitochondrial DNA deletions in mitochondrial myopathies. *Ann Neurol* 1990;**28**:94-7.

174 Larsson NG, Eiken HG, Boman H, Holme E, Oldfors A, Tulinius MH. Lack of transmission of deleted mtDNA from a woman with Kearns-Sayre syndrome to her child. *Am J Hum Genet* 1992;**50**:360-3.

175 Rotig A, Bessis JL, Romero N, *et al*. Maternally inherited duplication of the mitochondrial genome in a syndrome of proximal tubulopathy, diabetes mellitus, and cerebellar ataxia. *Am J Hum Genet* 1992;**50**:364-70.

176 Dunbar DR, Moonie PA, Swingler RJ, Davidson D, Roberts R, Holt IJ. Maternally transmitted partial direct tandem duplication of mitochondrial DNA associated with diabetes mellitus. *Hum Mol Genet* 1993;**2**:1619-24.

177 Ballinger SW, Shoffner JM, Gebhart S, Koontz DA, Wallace DC. Mitochondrial diabetes revisited. *Nat Genet* 1994;7:458-9.

178 Poulton J, Holt IJ. Mitochondrial DNA: does more lead to less? *Nat Genet* 1994;**8**:313-15.

179 Manfredi G, Vu T, Bonilla E, *et al*. Association of myopathy with large-scale mitochondrial DNA duplications and deletions: which is pathogenic? *Ann Neurol* 1997;**42**:180-8.

180 Poulton J, Deadman ME, Bindoff L, Morten K, Land J, Brown G. Families of mtDNA re-arrangements can be detected in patients with mtDNA deletions: duplications may be a transient intermediate form. *Hum Mol Genet* 1993;**2**:23-30.

181 Harding AE, Sweeney MG, Govan GG, Riordan-Eva P. Pedigree analysis in Leber hereditary optic neuropathy families with a pathogenic mtDNA mutation. *Am J Hum Genet* 1995;**57**:77-86.

182 Macmillan C, Kirkham T, Fu K, *et al*. Pedigree analysis of Leber's hereditary optic neuropathy families with the T14484C mtDNA mutation. *Neurology* 1997;**49**:A245.

183 Poulton J, Macaulay V, Marchington DR. Mitochondrial genetics '98: ss the bottleneck cracked? *Am J Hum Genet* 1998;**62**:752-7.

184 Chinnery PF, Howell N, Lightowlers RN, Turnbull DM. Genetic counseling and prenatal diagnosis of mtDNA disease. *Am J Hum Genet* 1998;**63**:1908-10.

185 Chinnery PF, Howell N, Lightowlers RN, Turnbull DM. MELAS and MERRF: the relationship between maternal mutation load and the frequency of clinically affected offspring. *Brain* 1998;**121**:1889-994.

186 Harding A, Holt I, Sweeney M, Brockington M, Davis M. Prenatal diagnosis of mitochondrial DNA $^{8993\ T>G}$ disease. *Am J Hum Genet* 1992;**50**:629-33.

187 Matthews PM, Hopkin J, Brown RM, Stephenson JB, Hilton-Jones D, Brown GK. Comparison of the relative levels of the 3243 (A→G) mtDNA mutation in heteroplasmic adult and fetal tissues. *J Med Genet* 1994;**31**:41-4.

188 Taylor RW, Chinnery PF, Clark KM, Lightowlers RN, Turnbull DM. Treatment of mitochondrial disease. *J Bioenerg Biomembr* 1997;**29**:195-205.

189 Gold R, Seibel P, Reinelt G, *et al*. Phosphorus magnetic resonance spectroscopy in the evaluation of mitochondrial myopathies: results of a 6-month therapy study with coenzyme Q. *Eur Neurol* 1996;**36**:191-6.

190 Eleff S, Kennaway NG, Buist NR, *et al*. 31P NMR study of improvement in oxidative phosphorylation by vitamins K3 and C in a patient with a defect in electron transport at complex III in skeletal muscle. *Proc Natl Acad Sci USA* 1984;**81**:3529-33.

191 Nishikawa K, Takahashi M, Yorifuji S. Long-term coenzyme Q10 therapy for a mitochondrial encephalomyopathy with cytochrome c oxidase deficiency: a $^{31}$P NMR study. *Neurology* 1989;**39**:339-43.

192 Bresolin N, Doriguzzi C, Ponzetto C, *et al*. Ubidecarenone in the treatment of mitochondrial myopathies: a multicenter double-blind trial. *J Neurol Sci* 1990;**100**:70-8.

193 Taivassalo T, De Stefano N, Matthews PM, *et al*. Aerobic training benefits patients with mitochondrial myopathies more than other chronic myopathies. *Neurology* 1997;**48**:A214.

194 Taylor RW, Chinnery PF, Turnbull DM, Lightowlers RN. Selective inhibition of mutant human mitochondrial DNA replication in vitro by peptide nucleic acids. *Nat Genet* 1997;**15**:212-15.

195 Shoubridge EA, Johns T, Karpati G. Complete restoration of a wild-type mtDNA genotype in regenerating muscle fibres in a patient with a tRNA point mutation and mitochondrial encephalomyopathy. *Hum Mol Genet* 1997;**6**:2239-42.

196 Clark K, Bindoff LA, Lightowlers RN, *et al*. Correction of a mitochondrial DNA defect in human skeletal muscle. *Nat Genet* 1997;**16**:222-4.